Data Article

Aberrant expression of CD19 in early T-cell precursor lymphoblastic leukemia- a diagnostic challenge

Abstract:

A systematic approach is required to diagnose acute leukemia. Most of the cases are satisfactorily diagnosed and categorized into subtypes. However, a few cases pose diagnostic dilemma secondary to immunophenotypic aberrancies which are defined as antigens that are normally restricted to a different lineage and expressed by a neoplastic population while absent from its normal non neoplastic counterpart. We report a rare case of Early T-cell Precursor Lymphoblastic Leukemia with aberrant expression of CD19. A 7-year-old boy referred to our hospital with his cervical lymph node biopsy reported as lymphoproliferative disorder. The patient was COVID-19 positive. Chest X-ray showed mild right sided pleural effusion with huge mediastinal mass. Flow cytometry on peripheral blood used to establish the diagnosis. The case is reported to improve knowledge regarding aberrant expression of markers. Hematopathology teams should be aware of this phenomenon so that appropriate workup can be done to reach correct diagnosis.

Key words: Acute leukemia, aberrancy, immunophenotype, flow cytometry, T-lineage

Introduction:

Aberrant expression is a phenomenon of abnormal expression or loss of expression of a lineage specific marker by a population of cells. These aberrancies occur due to genetic defects and have significant diagnostic and prognostic impact in hematologic malignancies [1]. In acute leukemia, these aberrancies play a crucial role in identification of minimal residual disease. WHO Classification of hematopoietic and lymphoid neoplasms 2017, clearly defined the criteria to assign a lineage [2]. The morphologically similar blast cells can be easily differentiated by immunophenotyping on the basis of expression of CD (cluster of differentiation) markers. Flow cytometry is the most efficient and sensitive method to determine the immunophenotype. Commonly expressed CD markers on T-cells include CD2, CD3, CD5, CD7 while CD19, CD20, CD22, CD79a are common markers expressed on B cells. CD3 is a pan-T cell marker expressed by T-lymphocytes throughout the maturation spectrum. CD19 is a lineage specific marker for B-cells and its expression in T-cell neoplasms is rarely found [3]. CD79a, another marker of B-lineage is reported to be aberrantly expressed in T-lymphoblastic leukemia (T-ALL) while CD19 expression is exceptionally rare in T-ALL. Co-expression of lineage specific markers impose a diagnostic challenge as these cases mimic Mixed Phenotype Acute Leukemia (MPAL). Therefore, knowledge regarding aberrancies is very important to establish a correct diagnosis. A small number of patients (3%) with double-positive CD7 and CD19 acute leukemia has been reported, in which the cellular and clinical characteristics of the disease have been analyzed [4]. To the best of our knowledge, co-expression of CD3 and CD19 is not a

frequent finding therefore the spectrum of disease is not studied in detail. We present a rare case of early T-cell precursor lymphoblastic leukemia with aberrant expression of CD19.

Case Presentation

A 7-year-old boy, presented to Pediatric Hematology-Oncology department with history of generalized body pain, weakness, facial puffiness, severe cough and orthopnea for 1-month and shortness of breath for last 10 days. On physical examination, he was febrile, tachycardiac, and tachypenic. There was cervical, axillary and inguinal lymphadenopathy and hepatomegaly. Chest X-ray showed mild right sided pleural effusion with huge mediastinal mass. Due to current situation of pandemic, sample for COVID-19 PCR was sent on admission and report was positive for COVID-19.

Before coming to The Indus hospital (TIH), Karachi, his initial workup done and reported as lymphoproliferative disorder on cervical lymph node biopsy. In TIH, on admission CBC revealed hemoglobin of 10.6 g/dL, total leukocyte count of 18.1×10^9/L with 30% blasts and platelet count of 270×10^9/L. Peripheral blood film showed small to medium sized blasts characterized by high nuclear to cytoplasmic ratio, fine nuclear chromatin, indistinct nucleoli and scant to moderate amount of pale basophilic non-vacuolated agranular cytoplasm (**Figure 1**).Coagulation profile and biochemistry including liver and renal function tests, magnesium, phosphorus, albumin, and alkaline phosphatase were within reference limits. LDH and D-dimer were raised, 2044 U/L and >4 ug/ml FEU respectively. There was mediastinal widening with bilateral air space opacities, on chest x-ray, and CT scan showed extensive infiltrating mediastinal mass consistent with history of lymphoma, extension of the disease process along the right pericardium and pleura observed. Large consolidation in the left lung was also noted. Few abdominal lymph nodes and bilateral renal deposits were also seen with mild hepatomegaly (**Figure 2**).

Immunophenotypic analysis of peripheral blood was requested by flow cytometry for further sub classification of lymphoproliferative disorder. (**Figure 3**) A significant blast population was identified by mononuclear gating and the expression of markers observed as mentioned below:

ICCD3 (+), CD7 (+), CD5 (Dim Partial+), sCD3 (-), CD4 (-), CD8 (-), TdT (-), CD1a (-), CD34 (+), CD99 (+), CD45 (+), CD38 (+), CD19 (+), HLA-DR (-), CD56 (-), Intracytoplasmic Myeloperoxidase (-), CD33 (-), CD13 (-)

The flow cytometric analysis revealed a very unsual finding i.e. expression of CD19 on a T-lymphoblast population. The bi-phenotypic expression of blasts raised suspicion of Mixed Phenotype Acute Leukemia (MPAL) as the expression of CD19 was repeated and rechecked. It was tested on two different flourochromes including PE CY7 and Per CP, both showed a bright positive expression of CD19. Rest of the immunophenotype of blast population was suggestive of Early-T-Cell Precursor lymphoblastic leukemia. As per WHO Classification of Hematopoietic

and Lymphoid Neoplasms 2017, further markers for B-lineage were tested again by flow cytometry and the results were as under:

CD79a (-), CD22 (-), CD20 (-) and CD10 (-)

On the basis of this extensive workup and considering the radiological findings, the case was concluded as Early T-cell Precursor lymphoblastic leukemia (ETP-ALL) with aberrant expression of CD19. The child was managed for ETP-ALL with upfront induction therapy based on modified Berlin-Frankfurt-Munster (BFM) protocol. After initial stabilization, COVID-19 treatment was also started to manage CIVID-19 related complications. However, patient expired within two weeks.

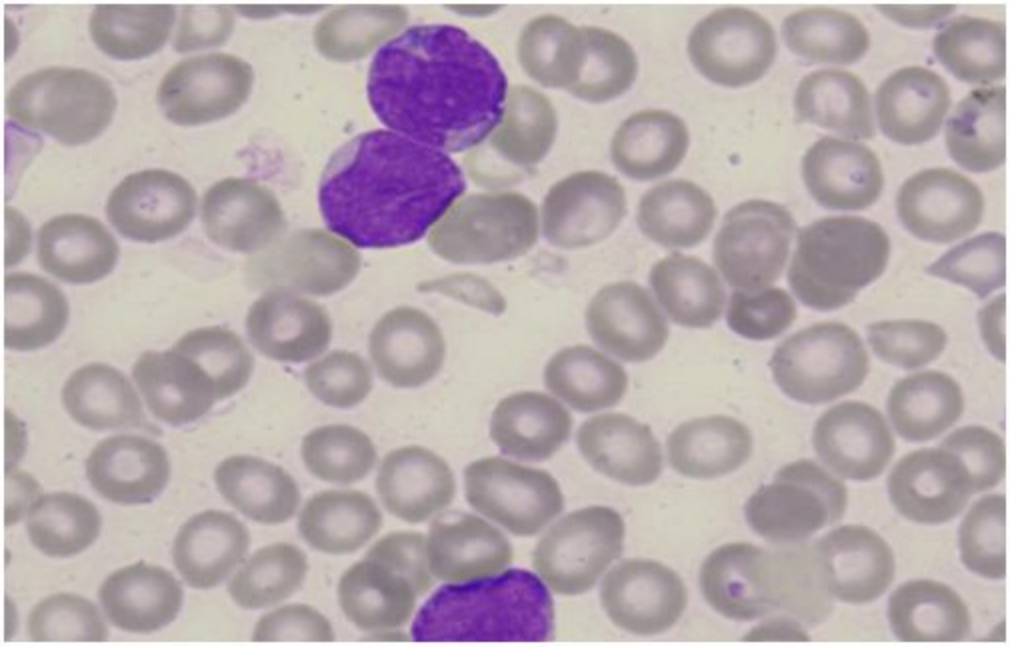

Figure1: Peripheral blood film exhibiting blasts

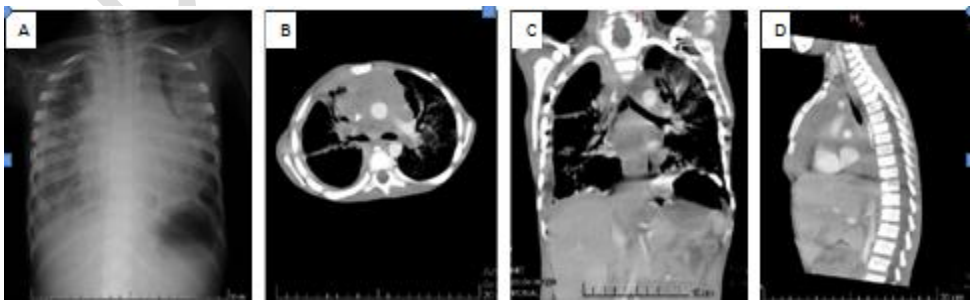

Figure 2: Frontal chest radiograph (A) showing bilateral widening of mediastinum. Axial (B), Coronal (C) and Sagittal (D) CT images demonstrate bilateral large heterogenous mediastinal mass occupying most of the anterior mediastinum.

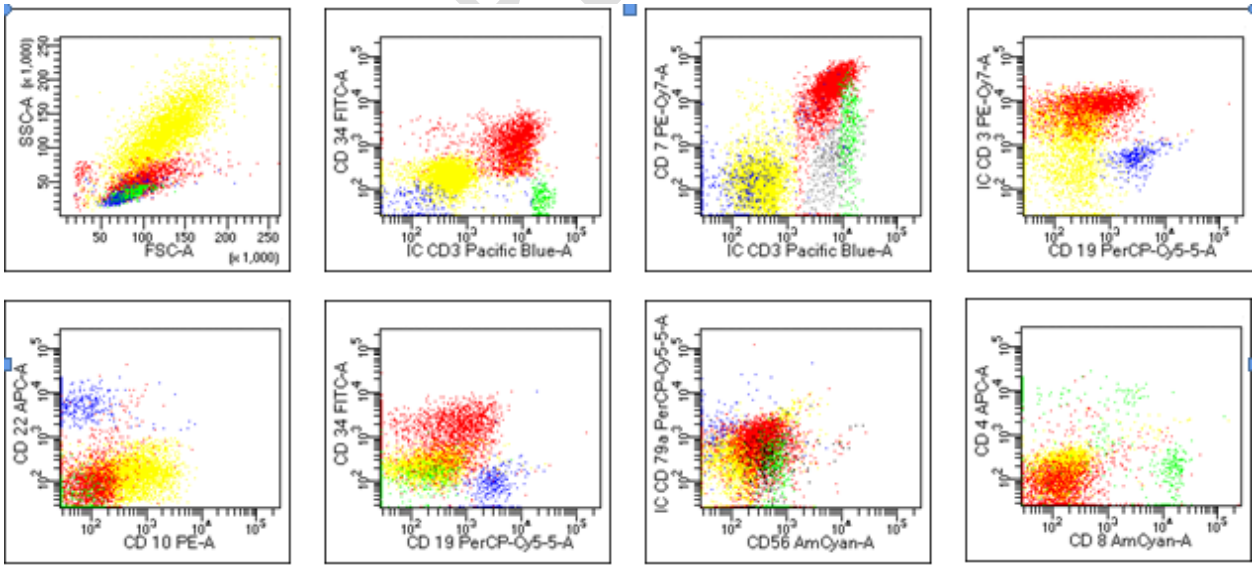

Figure 3: Immunophenotyping by Flowcytometry showing positivity for CD34, CD3, CD7 and CD19 (an aberrant expression).
Color Code: Red – T Lymphoblasts, Green – T Lymphocytes, Blue – B Lymphocytes, Yellow – Granulocytes.

Discussion:

The incidence of MPAL is relatively rare and the frequently reported subtypes are mostly B/Myeloid or T/Myeloid. B/T is the least common subtype in MPAL [5, 6]. The case presented above led a diagnostic challenge for our hematopathology team as the co-expression of two lineage specific markers led to initial suspicion of B/T MPAL. While after performing the extensive workup and analyzing the neoplastic population for all available markers pertinent to acute leukemia, we concluded the case as ETP-ALL with aberrant CD19 expression. Testing for co-expression and cross expression of markers require extensive panels to be tested in addition to fulfil the minimum diagnostic criteria. Hence it also impact the cost. Therefore, usually such panels are not in common practice in laboratories where cost is a major challeneg. In fact, flow cytometry is not a very common diagnostic modality in lower middle income countries and laboratories performing it very cautiously to cater all required markers in a wisely customized panels. We have 8-color flow cytometry so the aberrancy reported here in this particular case was identified in initial panel of our screening tube. However, repeat testing for confirmation as well as exclusion of other possibilities took multiple combination of markers to be tested.

Each marker has its own lineage specific role. For instance, physiologically CD3 helps in signal transduction [7]. Co-expression of B and T cells including CD7 and CD19 is reported in an elderly male while early T-cell precursor immunophenotype with aberrant expression of CD19 in a young boy is a very rare finding [4]. It's too early to comment on its prognostic significance but it is important to report such cases to share aberrancies observed which may lead to misdiagnosis.

As per WHO 2017, cytoplasmic CD3 is lineage specific marker for T cells. For B cells, multiple antigens are required for lineage confirmation of B cells. A strong CD19 expression along with strong expression of CD79a, cytoplasmic CD22 or CD10 is essential for B cell lineage [2]. If the expression of CD19 is weak, then two of the above markers are required to assign B-cell lineage. In present case, a single population of blasts identified with early T-cell precursor immunophenotype and strong positivity for CD19 which was confirmed using two different flourochromes. WHO criteria for MPAL not fulfilled as additional markers for B-cells were negative. On extensive search for similar reported cases, only 2-3 cases were found; out of those only one case is reported in pediatric age group [4, 8, 9]. Cytogenetic studies could not performed as patient was very sick due to ETP-ALL with large mediastinal mass and COVID-19 infection simultaneously. In the situation that a patient is positive for COVID-19, but requires urgent initiation of induction therapy, it is recommended to treat while monitoring vigilantly for COVID-19 symptoms and disease course. If symptoms develop, therapy should be discontinued and early initiation of cytokine modulators should be considered [10].

Though he responded well to ALL protocol initially but later he developed COVID related complications. Association of aberrant immunophenotype with disease course and prognosis cannot be determined by a single case. Cases with such aberrancies should not be classified as MPAL until the complete panel of markers is tested as per WHO recommendation.

Conclusion:

Aberrant immunophenotypes in hematological malignancies is a known but uncommon phenomenon. Furthermore, if present these phenotypes, which are actually a result of abnormal genetic programme, should be clearly delineate using stringent diagnostic criteria. CD19 expression in ETP-ALL is a diagnostic challenge but it may not be associated with aggressive form of disease with little or no response to standard treatment protocol. Further studies with larger numbers would help in validating and clarifying the prognostic role of such aberrant expression of lineage specific markers.

Consent: The parents of patient provided written informed consent to the publication of the case details.
Ethical Approval: Not applicable

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